AF
1637
JFW

USSN: 09/844,501
Dkt. No.: 8325-0015
S15-US1
PATENT

CERTIFICATE OF MAILING PURSUANT TO 37 CFR § 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Appeal Brief, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on **August 25, 2005**.

8/25/05 Michelle Hobson
Date Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Examiner: Jeffrey N. Fredman
WOLFFE et al.	Group Art Unit: 1637
Serial No.: 09/844,501	Confirmation No.: 9055
Filing Date: April 27, 2001	Customer No.: 20855
Title: DATABASES OF REGULATORY SEQUENCES; METHODS OF MAKING AND USING SAME	

TRANSMITTAL LETTER

Mail Stop Appeal Brief
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

Sir:

Transmitted herewith for filing, please find the following documents:

x Appeal Brief (19 pages) with attached Claims Appendix (5 pages), Evidence Appendix (8 pages) and Related Proceedings Appendix (1 page)

x Return receipt postcard

The fee is calculated as follows:

	NO. OF CLAIMS	CLAIMS PREVIOUSLY PAID FOR	EXTRA CLAIMS	RATE	FEE
Total Claims	30	- 122	0	x $50.00	$0
Independent Claims	2	- 22	0	x $200.00	$0
Multiple dependent claims not previously presented, add $360.00					$0
Total Amendment Fee					$0
Petition for Extension of Time					$0
Small Entity Reduction (if applicable)					$0
TOTAL FEE DUE					$0

The Commissioner is hereby authorized to charge any appropriate fees under 37 C.F.R. §§1.16, 1.17, and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 18-1648.

Respectfully submitted,

Date: August 25, 2005

By: 
Dahna S. Pasternak
Registration No. 41,411

ROBINS & PASTERNAK LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94303
Tel.: (650) 493-3400
Fax: (650) 493-3440

USSN 09/844,501
8325-0015
S15-US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

A.P. Wolffe *et al.*

Application No.: 09/844,501

Filed: April 27, 2001

For: DATABASES OF REGULATORY SEQUENCES; METHODS OF MAKING AND USING SAME

Examiner: Jeffrey N. Fredman

Group Art Unit: 1637

Confirmation No.: 9055

APPEAL BRIEF

ROBINS & PASTERNAK LLP
1731 Embarcadero Road
Suite 230
Palo Alto, CA 94303

Attorney for Appellant

TABLE OF CONTENTS

USSN 09/844,501
8325-0015
S15-US1

CLAIMS APPENIDIX

EVIDENCE APPENDICES:

(1) EXCERPT FROM "CURRENT PROTOCOLS IN MOLECULAR BIOLOGY" VOL. 2, eds. AUSUBEL, ET AL.

(2) EXCERPT FROM "GUIDE TO MOLECULAR CLONING TECHNIQUES," IN *METHODS IN ENZYMOLOGY,* VOL. 152, eds. BERGER & KIMMEL

(3) EXCERPT FROM OFFICE ACTION IN RELATED APPLICATION 10/083,682

RELATED PROCEEDINGS APPENDIX

USSN 09/844,501
8325-0015
S15-US1

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Alexandria, VA 22313 on **August 25, 2005**.

8/25/05 Michelle Hobson
Date Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

A.P. Wolffe *et al.*

Application No.: 09/844,501

Filed: April 27, 2001

For: DATABASES OF REGULATORY SEQUENCES; METHODS OF MAKING AND USING SAME

Examiner: Jeffrey N. Fredman

Group Art Unit: 1637

Confirmation No.: 9055

APPEAL BRIEF

Mail Stop Appeal Brief
Commissioner for Patents
Alexandria, VA 22313

Sir:

INTRODUCTION

Appellant submits one copy of this brief on appeal in accordance with Section 41.37 (69 Fed. Reg. 49962, Aug 2004). All claims were finally rejected under 35 U.S.C. § 103 in a Final Office Action mailed April 13, 2004. A Notice of Appeal was mailed August 9, 2004 and a Brief on Appeal was mailed on September 30, 2004. An Examiner's Answer was mailed on

November 17, 2004. Appellant's Reply to Examiner's Answer and Revised Reply to Examiner's Answers were mailed on January 18, 2005 and January 27, 2005, respectively. Pursuant to the "Order Returning Undocketed Appeal to Examiner," mailed July 12, 2005, which indicated that the Examiner's Answer should be vacated and an Appeal Brief resubmitted, Appellant submits this substitute Appeal Brief in compliance with 37 C.F.R. § 41.37. Pursuant to the Notification of Non-Compliant Brief mailed August 17, 2005, Appellant notes, as a courtesy to the Examiner, that the instant Appeal Brief contains different arguments than the brief submitted on September 30, 2004.

I. REAL PARTY IN INTEREST

Sangamo BioSciences, Inc., the assignee of record of the above-referenced patent application is the real party in interest in this matter.

II. RELATED APPEALS AND INTERFERENCES

Appellant has filed a Notice of Appeal (mailed June 29, 2005) in USSN 10/083,682, which is a CIP of the present application. No Appeal Brief has yet been filed, nor has a decision been rendered.

III. STATUS OF THE CLAIMS

Claims 123-152 are currently pending in the above-referenced case (hereinafter "the application"). The application was originally filed on April 27, 2001 with claims 1 to 122. Claims 1 to 122 were canceled and claims 123-152 were newly presented in a second preliminary amendment mailed July 23, 2002 and were variously amended in papers mailed December 17, 2003 and February 26, 2004. Following a telephone conference with the Examiner, Applicants amended the claims in a Supplemental Amendment mailed June 16, 2004, to make explicit what was previously implicit. These amendments were not entered.

Accordingly, claims 123-152 are pending as shown in the Claims Appendix. All pending claims remain rejected under 35 U.S.C. § 103.

IV. STATUS OF THE AMENDMENTS

In response to the Examiner's Final Office Action mailed April 13, 2004, Appellant filed a Response mailed on May 18, 2004. No amendments to the claims were made in that response. Following receipt of an Advisory Action mailed June 2, 2004, a telephone call was placed to the Examiner to discuss potential claim amendments and the Examiner encouraged Appellant to present those amendments. As a result, Appellant filed a Supplemental Response After Final, mailed on June 16, 2004, in which independent claims 123 and 143 were amended to make explicit that the claimed libraries or collections comprise different sequences. A second Advisory Action was mailed on June 29, 2004, indicating that amendments to the claims would not be entered. The claims were not amended in the Appeal Brief or in the Reply to Examiner's Answer. Thus, all claims remained rejected for the reasons set forth in the Final Office Action.

V. SUMMARY OF THE CLAIMED SUBJECT MATTER

The claimed subject matter relates to methods for preparing libraries of regulatory DNA sequences and to methods of isolating collections of regulatory polynucleotide sequences. The methods are based on the fact that functioning transcriptional regulatory sequences are present, in the cell, in a chromatin structure that differs from the chromatin structure of non-regulatory DNA (such as, for example, coding sequences). One manifestation of this difference in chromatin structure is that regulatory sequences are more susceptible to cleavage by nucleases (*i.e.*, more accessible) than is bulk chromatin, which is packaged into nucleosomal structures. Thus, exposure of cellular chromatin to nucleases generally results in preferential destruction of regulatory sequences relative to bulk genomic DNA.

Although it has been possible to identify regulatory sequences by their hypersensitivity to, *e.g.*, nucleases, it has not heretofore been possible to isolate and purify such sequences,

because they are destroyed in the process of being identified. The presently claimed subject matter provides, for the first time, methods not only for identifying, but also for isolating and purifying these regulatory sequences. Moreover, the claimed methods allow the simultaneous purification of a plurality of regulatory sequences from a cell, making it possible to obtain DNA libraries containing all of the regulatory DNA sequences that are active in a particular cell type.[1]

In particular, the methods of preparing a library typically comprise providing a cell nucleus comprising cellular chromatin (page 44, lines 13-14; page 102, lines 15-18; page 112, line 33 through page 113, line 10); contacting the nucleus with a first enzyme that reacts with accessible regions of cellular chromatin (page 30, lines 12-17; page 32, lines 18-22; page 102, lines 30-33; page 113, line 11); deproteinizing the cellular chromatin to generate deproteinized DNA (page 44, line 29 through page 45, line 2; page 49, lines 27-29; page 113, lines 14-16); contacting the deproteinized DNA with a second enzyme to generate DNA fragments (page 49, lines 27-29; page 113, lines 18-22); contacting the DNA fragments with a population of vector molecules, the vector molecules comprising a first end that is compatible with the first enzyme and a second end that is compatible with the second enzyme, under conditions favorable to ligation of compatible ends (page 50, lines 1-4; page 113, line 28 through page 114, line 5); and selecting polynucleotides comprising a DNA fragment ligated to a vector molecule (pages 50-51; page 114, lines 5-7). *See* also page 52, lines 3-15. Furthermore, during certain steps in the method, a cell nucleus can be embedded in agarose (page 49, lines 29-31; page 113, lines 12-24).

The cells from which the claimed libraries can be isolated include animal cells, plant cells and microbial cells. (page 56, line 2). In certain embodiments, a plurality of different libraries of regulatory DNA sequences are prepared, wherein each library is obtained from a different cell. The libraries may be obtained from cells at different stages of development; cells in different tissues; diseased cells and counterpart normal cells; cells that express a gene of interest at

[1] This is in some ways analogous to microarray analyses, which identify all of the transcribed sequences (*i.e.*, expressed genes) in a cell. The presently claimed methods identify all of the regulatory sequence (responsible for the transcription of those expressed genes) in the cell.

different levels; and/or infected cells and counterpart uninfected cells (page 7, lines 31-34; page 47, lines 5-9).

In any of these methods, the first enzyme may a nuclease, such as DNAse I (page 48, line 7; page 102, lines 30-33) or a restriction enzyme (page 32, lines 18-20; page 113, line 11). In addition, a plurality of different libraries of regulatory DNA sequences may be prepared and, for each library, a different first enzyme is used (page 7, lines 29-31). The different libraries may be combined (page 47, lines 10-11). In certain embodiments, the DNaseI ends are converted to blunt ends (page 51, lines 3-6). Similarly, the second enzyme may be a restriction enzyme, such as Sau3AI (page 49, lines 27-29; page 50, lines 4-8; page 113, lines 18-22). In certain embodiments, the second end of the vector molecule is generated by digestion with BamHI (page 50, lines 1-5; page 113, line 31 through page 114, line 2). The first end of the vector may be blunt-ended, for example as generated by digestion with EcoRV or SmaI (page 50, lines 1-7).

The claimed subject matter also relates to a method for isolating a collection of polynucleotides comprising cellular regulatory sequences, wherein the method comprises: contacting cellular chromatin with a probe, wherein the probe reacts with accessible regions of cellular chromatin (page 4, lines 27-30); subsequently fragmenting the cellular chromatin to generate a collection of polynucleotide fragments (page 4, lines 30-34); and selectively cloning polynucleotide fragments that comprise a site of probe reaction (page 28, lines 6-7; page 50, lines 1-7). In certain embodiments, reaction of the probe with cellular chromatin results in polynucleotide cleavage at the site of reaction (page 5, line 29; page 49, lines 23-24).

The cellular chromatin may be present in an isolated nucleus (page 44, lines 13-14) and, during certain steps of the method, the isolated nucleus may be embedded in agarose (page 52, lines 8-10). The probe used may be an enzyme, for example a nuclease (page 4, lines 27-28; page 5, line 29) such as DNAseI (page 26, line 32; page 49, lines 25-27) or a restriction enzyme (page 32, lines 18-20; page 52, lines 5-6; page 113, line 11). Fragmentation can be achieved by the use of a restriction enzyme (page 49, lines 27-29) such as Sau3AI (page 50, lines 4-8; page 52, line 12; page 113, lines 18-22).

VI. GROUNDS OF REJECTION

1. Claims 123-152 stand rejected under 35 U.S.C. 103(a) as being obvious over U.S. Patent No. 5,635,355 (hereinafter "Grosveld"), either alone or in combination with the NEB Catalog, U.S. Patent No. 5,500,356 or U.S. Patent No. 6,444,421.

VII. ARGUMENTS

1. The Claim Term "Library" Has Been Improperly Construed by the Examiner

In rejecting the pending claims as obvious over Grosveld, the Examiner has failed to consider the claimed method steps, instead fixing on the term "library" in the preamble of claim 123 and misconstruing this term. In particular, the remaining obviousness rejections over U.S. Patent No. 5,635,355 (hereinafter "Grosveld" or "the primary reference"), alone or in combination with various secondary references, are based on the Examiner's contention that a library can comprise any "cloned set of nucleic acids" apparently including multiple copies of the same cloned nucleic acid. *See, e.g.,* Final Office Action, mailed April 13, 2004, at pages 9-10. The Examiner attempts to support this strained interpretation of the term "library" by citing a single sentence at page 46 of the specification, which states "the term "library" refers to a pool of DNA fragments that have been propagated in some type of a cloning vector." *Id.*

Appellant's position has been, and remains, that the plain meaning of this claim term would be understood by one of skill in the art to refer to a collection of different polynucleotides. Furthermore, both the intrinsic evidence of the specification and file history, as well as the extrinsic evidence of record, are entirely consistent with this plain meaning in the field of molecular biology. Thus, the Examiner's interpretation of the term "library" is inconsistent with the plain meaning of the term, the intrinsic evidence and the extrinsic evidence of record.

For the reasons of record and as recently clarified in the Federal Circuit's *en banc* decision in *Phillips v. AWH,* 03-1269, -1286 (July 12, 2005) (Fed. Cir. 2005), Appellant submits that the plain and customary meaning of the term "library" to the skilled artisan clearly refers to a collection of different polynucleotides. Accordingly, because the term "library" must be

construed to refer to a collection of different polynucleotide sequences, Grosveld (which discloses multiple copies of the same sequence; *i.e.*, a clone) is not relevant to the claims on appeal and the obviousness rejections cannot stand.

a) The Term "Library" Must Be Given Its Customary Meaning, As Determined by a Person of Skill in the Art

As noted in *Phillips,* construction of any claim term must begin with the claims themselves. *Phillips,* at pages 8-13. According to Philips, and to a wealth of prior case law[2], "the ordinary and customary meaning of a claim term is the meaning that term would have to a person of ordinary skill in the art in question at the time of the invention." *Id.* at page 9. Simply put, "the inquiry into how a person of ordinary skill in the art understands a claim term provides an objective baseline from which to begin claim interpretation." *Id.* at page 9.

The terms "clone" and "library" are among those used in the art of molecular biology to describe different types of recombinant DNA. As is well-known to molecular biologists, the term "clone" refers to a collection of DNA molecules of identical sequence; obtained, for example, by ligating a restriction fragment into a vector. *See* Figure 1 on following page. On the other hand, the term "library" is understood to denote a collection of DNA molecules having different sequences; obtained, for example, by digesting genomic DNA with a restriction enzyme and ligating the resulting collection of genomic restriction fragments into a vector. *See* Figure 2 on the following page.[3]

[2] For example, *Vitronics Corp. v. Conceptronic, Inc.* 90 F.3d 1576 (Fed. Cir. 1996); *Innova/Pure Water, Inc. v. Safari Water Filtration Systems, Inc.* 381 F.3d 1111 (Fed. Cir. 2004)

[3] To the extent that the examiner interprets the term "library" to include a collection of DNA molecules, each of which consists of a vector with multiple inserts, Appellants note that such a collection is a clone, not a library, since the sequences of all DNA molecules in such a collection are identical. *See* Figure 3.

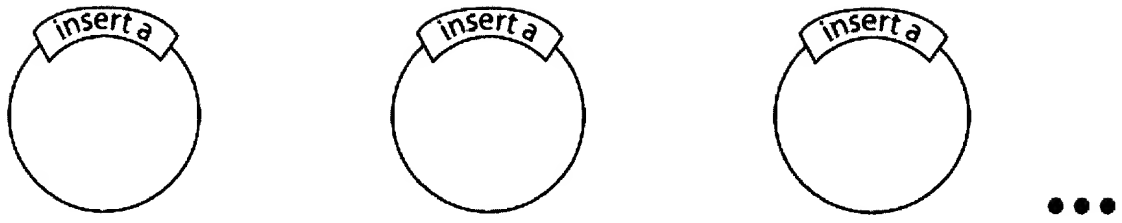


Figure 1. Schematic diagram of a clone.

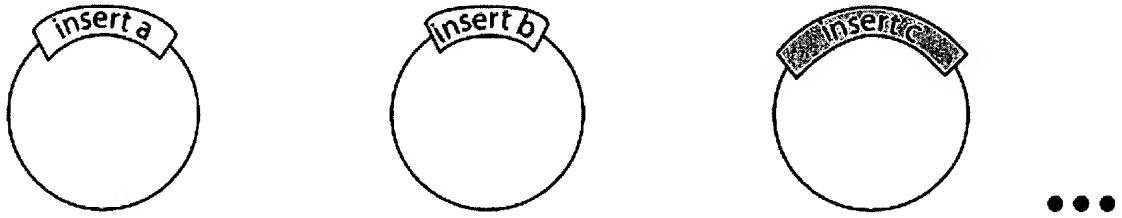


Figure 2. Schematic diagram of a library.

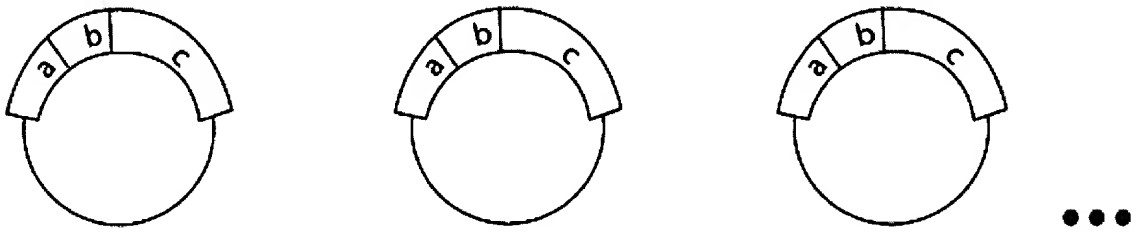


Figure 3. Schematic diagram of a clone containing multiple inserts.

In addition, and as approved by the Federal Circuit in *Phillips* (*Id.* at page 12.), the context in which the term "library" is used in the claims is entirely consistent with its plain meaning to one of skill in the art. In particular, independent claim 123 refers to a plurality of DNA fragments and a plurality of regulatory sequences. Moreover, the claims require that these multiple sequences be obtained from the cellular chromatin contained in a cell nucleus. As such, these sequences must be different from each other. Indeed, how could multiple fragments obtained from the nucleus of a single cell all be identical?

Based on how the term "library" is used in the claims, it is clear that a skilled artisan would understand that the ordinary and plain meaning of the term "library" is a collection (or pool) of different DNA sequences.

b) The Intrinsic Evidence is Consistent with the Plain Meaning of the Term "Library"

(i)Appellant's "Definition" of the Term "Library" in the Body of the Specification Does Not Contradict the Plain Meaning

As further set forth in *Phillips,* "the claims, of course, do not stand alone. Rather, they are part of a 'fully integrated written instrument' [citing *Markman*]. For that reason, claims 'must be read in view of the specification, of which they are a part.' " *Phillips,* at page 13. Nonetheless, the court in *Phillips* restates the axiom that only in cases in which the specification contains a "special definition given to the claim term ... that differs from the meaning it would otherwise possess" can the plain and customary meaning as determined from the claim language be overridden. *Id.* at page 16.

Here, the Examiner has repeatedly asserted that the sentence on page 46 of the specification (*i.e.,* "the term "library" refers to a pool of DNA fragments that have been propagated in some type of a cloning vector") somehow contradicts the plain meaning of

"library" as used in the claims to refer to a collection of different polynucleotides, as it would be understood by the skilled artisan. This assertion is misplaced and is not supported by the record. Even when taken in isolation, the so-called "definition" on page 46 of the specification actually supports the interpretation of the term "library" to include multiple different fragments inasmuch as a "pool" of fragments would be understood by the skilled artisan as another way of saying a collection of different fragments. Therefore, as would be understood by the skilled artisan, the definition on page 46 is entirely consistent with the plain and customary meaning of the term "library."

Not only does the single sentence cited by the Examiner fail to support the assertion that Appellants intended to override the plain meaning of the term "library" (*i.e.,* to refer to multiple copies of the same fragment), the Examiner has also erred in viewing the single sentence of the specification in isolation. Passages of the specification, like the claims, must be viewed in context. *Phillips,* at pages 13-16. As noted above and previously, the specification as a whole makes it abundantly clear that a claimed library is made up of a pool of different DNA fragments. Appellant has repeatedly pointed to multiple locations in the specification supporting this, for example by the fact that terms referring to components of the claimed libraries are used in the plural. *See, e.g.,* page 28, lines 3-11; page 38, lines 20-24; page 45, lines 7-10; and page 47, lines 5-6 and lines 15-17. For instance, on page 46, lines 6-7, it is stated that "As used herein, the term "library" refers to a pool of DNA fragments that have been propagated in some type of a cloning vector." In addition, page 47, lines 5-6 states that "The libraries formed can represent accessible regions for a particular cell type or cellular condition," while page 45, lines 7-8 states that "Collections of accessible region sequences from a particular cell can be cloned to generate a library ..." Plainly, Appellant uses terms such as "pool," "fragments," and "accessible regions" throughout the specification in the plural form to indicate that a library includes a plurality of nucleotide sequences that are different from one another.

The working examples provided in the specification also show libraries containing a collection of different polynucleotide sequences. *See, e.g.,* Example 15, pages 112-116. Given

that the specification makes clear throughout that the claimed libraries necessarily include a plurality of DNA sequences obtained from a single genome; it is incumbent upon the Examiner to set forth, based upon factual evidence (not unsupported assertions), precisely how the multiple sequences contained by such libraries could possibly be identical. In reality, the claims and specification make it plain that the claimed subject matter comprises collections of different sequences.

(ii) The Term "Library" Must Also Be Construed In Light of the Prosecution History

The Federal Circuit in *Phillips* has also reiterated that the prosecution history, like the specification and claims themselves, is part of the "intrinsic evidence" and, accordingly, provides evidence of how the inventor understood the claims. *Phillips* at page 17.

Here, the Examiner has improperly ignored the fact that the prosecution history establishes that Appellant understood the term "library" to mean a collection of polynucleotides comprising different sequences, consistent with its plain meaning to one of skill in the art.[4] Specifically, Appellant has repeatedly noted in his Responses that the term "library" was known in the art, at the time of filing, to refer to a collection of different polynucleotides. Moreover, Appellant affirmatively amended the claims to make this explicit. *See,* Supplemental Response filed June 16, 2004. Appellant reiterates herein the fact that this amendment did not raise new issues, but merely clarified an already-defined claim term.

In any event, although the Office refused to enter this amendment, Appellant submits that this un-entered amendment, along with the repeated statements during prosecution that the library comprises a plurality of different DNA fragments, is part and parcel of the intrinsic evidence, unambiguously demonstrating that the term "library" cannot be construed as the Examiner contends to include multiple DNA molecules, each containing the same sequence.

[4] *See* Response dated December 17, 2003 (resubmitted February 26, 2004) at pp. 6-8; Response dated May 18, 2004 at pp. 2-3 and Exhibits A and B attached thereto; and Supplemental Response After Final dated June 16, 2004 at page 6

In sum, all the intrinsic evidence -- claims, specification and prosecution history -- establish that the term "library" refers to clones of multiple, different DNA sequences.

c) The Meaning of the Term "Library" Found in Extrinsic Evidence Is Entirely Consistent With the Plain Meaning and the Intrinsic Evidence

The court in *Phillips* also reaffirmed that the construction of a claim term using intrinsic evidence may be supplemented by the use of extrinsic evidence, including all evidence external to the patent and prosecution history. *Phillips* at page 18ff. The usefulness of dictionary and treatise definitions was characterized by the Court as follows (*Id.* at pages 18-19):

> Within the class of extrinsic evidence, the court has observed that dictionaries and treatises can be useful in claim construction [citation omitted]. We have especially noted the help that technical dictionaries may provide to a court "to better understand the underlying technology" and the way in which one of skill in the art might use the claim terms. [citation omitted]. Because dictionaries, and especially technical dictionaries, endeavor to collect the accepted meanings of terms used in various fields of science and technology, those resources have been properly recognized as among the many tools that can assist the court in determining the meaning of particular terminology to those of skill in the art of the invention.

In the case on appeal, Appellant has submitted dictionary definitions and treatises available at the time of filing that support the construction of the claim term "library" to mean a collection of different polynucleotides. These definitions provide further proof (if any is required) that it was well-known in the art of molecular biology, at the time of filing, that a nucleic acid library is a collection of different nucleotide sequences. *See*, for example, Ausubel *et al.* (1987), Exhibit A attached to Response dated May 18, 2004 (Evidence Appendix (1) hereto):

> . . . a recombinant DNA library consists of a large number of recombinant DNA clones, *each one of which contains a different segment of foreign DNA.* (emphasis added)

See also Berger *et al.* (1987), Exhibit B attached to Response dated May 18, 2004 (Evidence Appendix (2) hereto):

> A library is a mixture of clones constructed by inserting either cDNA or fragments of genomic DNA into a suitable vector. The term *library* implies the existence of large numbers of different recombinants . . . (emphasis in original)

Thus, extrinsic evidence, in the form of dictionaries and treatises, fully corroborates both the plain meaning and the intrinsic evidence in demonstrating that a library is a collection of different sequences, not simply multiple copies of the same sequence.

Appellant also notes that, in the related application USSN 10/083,682 (a continuation-in-part of the present application), the Office has defined the term "library" as follows (*see,* USSN 10/083,682: Office Action dated September 9, 2004, page 6 (copy attached as Evidence Appendix (3)):

> . . . a library is interpreted as "an unordered collection of clones (i.e., cloned DNA from a particular organism)" [citation omitted]. Thus, the number of clones of polynucleotides in each library may vary.

In summary, the extrinsic evidence provided by Appellant does not, in any way, contradict either the plain meaning or the intrinsic evidence, with respect to the meaning of the term "library." Rather, the "extrinsic evidence" entirely supports the intrinsic evidence establishing that a library contains clones of different DNA sequences. Furthermore, in view of the consistency of the definition of "library" as between intrinsic and extrinsic evidence, any judicial concerns that extrinsic evidence may be less reliable than intrinsic evidence are alleviated.

The intrinsic and extrinsic evidence in this case also demonstrates that the Examiner's construction of the term "library" to include clones of the same sequences is artificial. In reality, the Examiner's interpretation of the claim term "library" is entirely inconsistent with the ordinary meaning of the term, the intrinsic evidence of the specification and prosecution history,

and the meaning given in dictionaries, treatises and the like (extrinsic evidence). When properly interpreted using intrinsic evidence alone or intrinsic evidence supported by extrinsic evidence, the claims on appeal cannot be obvious over a reference that fails to disclose a collection of different DNA sequences. The Examiner has provided no evidence, either intrinsic or extrinsic, to support his erroneous claim construction, relying merely on his unsupported opinion.

2. *Prima facie* obviousness of claims 123-152 has not been established

When the claims are accurately construed, it is clear that the claims are non-obvious over the cited reference(s). In particular, the primary reference Grosveld does not teach or suggest clones of different polynucleotide sequences as claimed. Rather, Grosveld teaches, in one section, the mapping of DNase hypersensitive sites and, in another section, the construction of a single clone containing four fragments, each of which comprises a sequence that is DNase hypersensitive in a particular cell type. Nowhere does Grosveld disclose or suggest the construction of a library comprising a collection of clones, each representing a different hypersensitive site.

In fact, there are absolutely no teachings by Grosveld regarding preparation of libraries in any way whatsoever. Rather, Grosveld states that hypersensitive sites may be "mapped" (col. 7, lines 59-63). The only cloning referred to in Grosveld involved construction of a plasmid containing sequences that, in certain cells, comprise DNase hypersensitive sites. Cloning of such sequences into a vector results in the production of multiple copies of the same construct, or what is normally referred to in the art as a "clone." Cloning as described in Grosveld does not produce a library of polynucleotide sequences as claimed, in the same way that a building containing multiple copies of the same book for loan would not be considered a library.

Moreover, it is again noted that, since Grosveld's disclosure is directed to obtaining integration site-independent gene expression using DNase hypersensitive sites, it is aimed at a different problem than that of the presently-claimed subject matter, which is directed to genome-wide isolation and purification of regulatory sequences. Previously, regulatory sequences could

not be isolated because they were destroyed in the process of being identified. Accordingly, there is no motivation for one of skill to look to Grosveld for guidance on how to simultaneously isolate and purify a plurality of regulatory sequences from a cell.

Thus, the fact remains that Grosveld merely discloses a clone, not a library, and thus there is no *prima facie* case for the obviousness of the claimed methods for constructing libraries.

(a) Grosveld Does Not Teach The Claimed Methods

Appellant also notes that Grosveld fails to teach the claimed method steps. Specifically, col. 8, lines 1-21 of Grosveld fails to teach or suggest steps (a)-(d) of claim 123. Whereas steps (a)-(d) require deproteinization between use of the first and second enzymes, Grosveld teaches that proteinase K digestion is performed after exposure to both DNAseI (which the Examiner asserts is Grosveld's equivalent of the first enzyme of the claims) and various restriction enzymes (which the Examiner asserts is Grosveld's equivalent of the second enzyme of the claims). *See, also,* Response mailed December 17, 2003 at pp. 6-8.

Furthermore, with respect to steps (e) and (f), Appellant reiterates that Grosveld does not teach or suggest the selective cloning of these steps. Instead, Grosveld teaches, at col. 15, that the "fragments" that are used for cloning are made by digesting DNA with two restriction enzymes whose recognition sites flank a DNase hypersensitive site (*i.e.*, the fragments cloned by Grosveld encompass a DNase hypersensitive site). Thus, unlike the claimed methods, in which the fragments which are cloned have a first end which resided in an accessible region (Claim 123, step b), Grosveld teaches the cloning of fragments having both ends outside an accessible region (in his case, a DNase hypersensitive site).

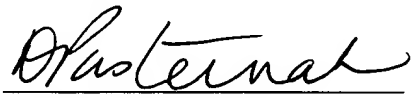
Thus, for the reasons of record, Appellant reiterates that Grosveld does not teach or suggest the manipulative steps set forth in the claims on appeal. Accordingly, a *prima facie* case of obviousness has not been and cannot be established based on Grosveld.

CONCLUSION

For the reasons stated above, Appellant respectfully submits that the pending claims are patentable over the art cited by the Examiner. Accordingly, Appellant requests that the rejections of the claims on appeal be reversed, and that the application be remanded to the Examiner so that the appealed claims can proceed to allowance.

Respectfully submitted,

Date: August 25, 2005

By: 

Dahna S. Pasternak
Registration No. 41,411
Attorney for Appellant

ROBINS & PASTERNAK LLP
1731 Embarcadero Road, Suite 230
Palo Alto, CA 94303
Telephone: (650) 493-3400
Facsimile: (650) 493-3440

CLAIMS APPENDIX

CLAIMS ON APPEAL

1 to 122. (canceled).

123. (previously presented): A method for preparing a library of regulatory DNA sequences from a cell, the method comprising:

(a) providing a cell nucleus, wherein the nucleus comprises cellular chromatin;

(b) contacting the nucleus with a first enzyme, wherein the first enzyme reacts with accessible regions of cellular chromatin;

(c) deproteinizing the cellular chromatin to generate deproteinized DNA;

(d) contacting the deproteinized DNA with a second enzyme to generate DNA fragments;

(e) contacting the DNA fragments obtained in step (d) with a population of vector molecules, wherein the vector molecules comprise a first end that is compatible with the first enzyme and a second end that is compatible with the second enzyme, under conditions favorable to ligation of compatible ends; and

(f) selecting polynucleotides comprising a DNA fragment ligated to a vector molecule.

124. (previously presented): The method of claim 123, wherein the cell is selected from the group consisting of animal cells, plant cells and microbial cells.

125. (previously presented): The method of claim 123, wherein the first enzyme is a nuclease.

126. (previously presented): The method of claim 125, wherein the nuclease is DNase I.

127. (previously presented): The method of claim 125, wherein the nuclease is a restriction enzyme.

128. (previously presented): The method of claim 123, wherein the second enzyme is a restriction enzyme.

129. (previously presented): The method of claim 128, wherein the restriction enzyme is Sau3A I.

130. (previously presented): The method of claim 129, wherein the second end of the vector molecule is generated by digestion with BamH I.

131. (previously presented): The method of claim 126, wherein, subsequent to step (b), the DNase I ends are converted to blunt ends.

132. (previously presented): The method of claim 131, wherein the first end of the vector molecule is a blunt end.

133. (previously presented): The method of claim 132, wherein the first end of the vector molecule is generated by digestion with EcoRV or SmaI.

134. (previously presented): The method of claim 123 wherein, during steps (b) – (d), the nucleus is embedded in agarose.

135. (previously presented): The method of claim 123, wherein a plurality of different libraries of regulatory DNA sequences are prepared, wherein each library is obtained from a different cell.

136. (previously presented): The method of claim 135 wherein, in step (a), nuclei are obtained from cells at different stages of development.

137. (previously presented): The method of claim 135 wherein, in step (a), nuclei are obtained from cells in different tissues.

138. (previously presented): The method of claim 135 wherein, in step (a), nuclei are obtained from diseased cells and counterpart normal cells.

139. (previously presented): The method of claim 135 wherein, in step (a), nuclei are obtained from infected cells and counterpart uninfected cells.

140. (previously presented): The method of claim 135 wherein, in step (a), nuclei are obtained from cells that express a gene of interest at different levels.

141. (previously presented): The method of claim 123, wherein a plurality of different libraries of regulatory DNA sequences are prepared and, for each library, a different first enzyme is used.

142. (previously presented): The method of claim 141, wherein the different libraries are combined.

143. (previously presented): A method for isolating a collection of polynucleotides comprising cellular regulatory sequences, wherein the method comprises:

(a) contacting cellular chromatin with a probe, wherein the probe reacts with accessible regions of cellular chromatin;

(b) subsequently fragmenting the cellular chromatin to generate a collection of polynucleotide fragments; and

(c) selectively cloning polynucleotide fragments of step (b) comprising a site of probe reaction.

144. (previously presented): The method of claim 143, wherein reaction of the probe with cellular chromatin results in polynucleotide cleavage at the site of reaction.

145. (previously presented): The method of claim 143, wherein the cellular chromatin in present in an isolated nucleus.

146. (previously presented): The method of claim 145 wherein, in steps (a) and (b), the isolated nucleus is embedded in agarose.

147. (previously presented): The method of claim 143, wherein the probe is an enzyme.

148. (previously presented): The method of claim 147, wherein the enzyme is a nuclease.

149. (previously presented): The method of claim 148, wherein the nuclease is a restriction enzyme.

150. (previously presented): The method of claim 148, wherein the nuclease is DNase I.

151. (previously presented): The method of claim 143 wherein, in step (b), cellular chromatin is fragmented by restriction enzyme digestion.

152. (previously presented): The method of claim 151, wherein the restriction enzyme is Sau3A1.

EVIDENCE APPENDIX

Three (3) documents are submitted with this appendix:

Evidence Appendices (1) and (2): EXCERPT FROM "CURRENT PROTOCOLS IN MOLECULAR BIOLOGY" VOL. 2, eds. AUSUBEL, ET AL and EXCERPT FROM "GUIDE TO MOLECULAR CLONING TECHNIQUES," IN *METHODS IN ENZYMOLOGY,* VOL. 152, eds. BERGER & KIMMEL were submitted by Appellant with a Response mailed May 18, 2004, which was responsive to a Final Office Action mailed April 13, 2004. The Advisory Action mailed June 6, 2004 indicated that the Examiner considered the request for reconsideration (Box 5 of Advisory Action mailed June 6, 2004), which included Evidence Appendices (1) and (2).

Evidence Appendix (3): EXCERPT FROM OFFICE ACTION IN RELATED APPLICATION 10/083,682 was submitted by Appellant with the Brief of Appeal on mailed September 30, 2004 and was addressed by the Examiner on page 13 of the Examiner's Answer, mailed September 17, 2004.

CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

VOLUME 2

EDITED BY

Frederick M. Ausubel
Department of Molecular Biology, Massachusetts General Hospital
Department of Genetics, Harvard Medical School

Roger Brent
Department of Molecular Biology, Massachusetts General Hospital
Department of Genetics, Harvard Medical School

Robert E. Kingston
Department of Molecular Biology, Massachusetts General Hospital
Department of Genetics, Harvard Medical School

David D. Moore
Department of Molecular Biology, Massachusetts General Hospital
Department of Genetics, Harvard Medical School

J. G. Seidman
Department of Genetics, Harvard Medical School

John A. Smith
Departments of Molecular Biology and Pathology, Massachusetts General Hospital
Department of Pathology, Harvard Medical School

Kevin Struhl
Department of Biological Chemistry, Harvard Medical School

Published by Greene Publishing Associates and Wiley-Interscience

John Wiley & Sons

New York • Chichester • Brisbane - [illegible] - [illegible]

Library of Congress Cataloging in Publication Data:

Current protocols in molecular biology. 2 vols.
1. Molecular biology—Technique. 2. Molecular biology—Laboratory manuals. I. Ausubel, Frederick M.

QH506.C87 1987 574.8'8'028 87-21033
ISBN 0-471-50338-X
ISBN 0-471-50337-1 (Vol. 2 binder)

Printed in the United States of America

10 9 8 7 6 5 4

Core + Supps. 1, 2, & 3

INTRODUCTION

The usual approach to isolating a recombinant DNA clone encoding a particular gene or mRNA sequence is to screen a recombinant DNA library. As described in Chapter 5, a recombinant DNA library consists of a large number of recombinant DNA clones, each one of which contains a different segment of foreign DNA. Since only a few of the thousands of clones in the library encode the desired nucleic acid sequence, the investigator must devise a procedure for identifying the desired clones. The optimal procedure for isolating the desired clone involves a positive selection for a particular nucleic acid sequence. If the desired gene confers a phenotype that can be selected in bacteria, then only the desired clone will grow under selective conditions, and it can be isolated in a rapid, effortless fashion. However, most eukaryotic genes and even many bacterial sequences do not encode a gene with a selectable function. Clones encoding nonselectable sequences are identified by screening libraries: the desired clone is identified either because it hybridizes to a nucleic acid probe or because it expresses a segment of protein that can be recognized by an antibody.

Screening libraries involves the development of a rapid assay to determine whether a particular clone contains the desired nucleic acid sequence. This assay is used first to identify the recombinant DNA clone in the library and then to purify the clone (see Fig. 6.0.1). Normally, this screening procedure is performed on bacterial colonies containing plasmids or cosmids or on bacteriophage plaques. To test a large number of clones at one time, the library is spread out on agarose plates (*UNIT 6.1*), then the clones are transferred to filter membranes (*UNIT 6.2*). The clones can be simultaneously hybridized to a particular probe (*UNITS 6.3* and *6.4*) or bound to an antibody (*UNIT 6.7*). When the desired clone is first identified, it is usually found among many undesirable clones; an important feature of library screening is the isolation of the desired clones (*UNITS 6.5* and *6.6*). Another method for identifying the desired clone involves hybrid selection (*UNIT 6.8*), a procedure in which the clone is used to select its mRNA. This mRNA is characterized by its translation into the desired protein.

To screen a DNA library, one must first devise the screening procedure. The next important choice is the selection of a recombinant DNA library. When choosing which library to screen the investigator should consider whether he or she wants to isolate clones encoding the gene or the mRNA sequence. cDNA clones will encode the mRNA

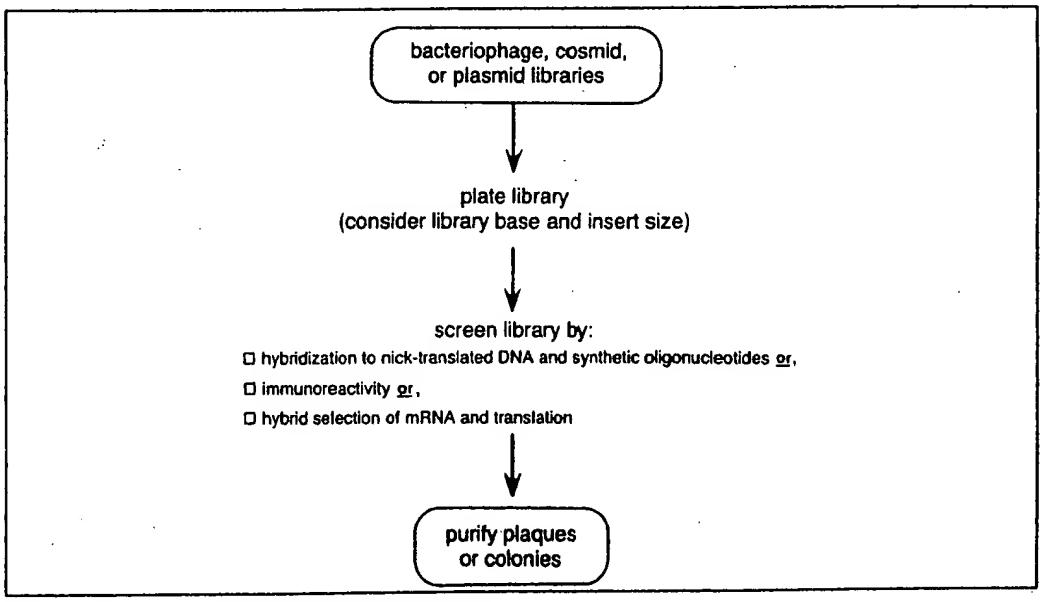


Figure 6.0.1 Flow chart for screening libraries.

Methods in Enzymology

Volume 152

Guide to Molecular Cloning Techniques

EDITED BY

Shelby L. Berger

NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

Alan R. Kimmel

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

1987

ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
Orlando San Diego New York Austin
Boston London Sydney Tokyo Toronto

plaques *in situ* to replace those that have been removed during the preparation of filters or to increase subsequent hybridization signals.

1. Prepare an overnight culture of plating bacteria (this volume [13 or 17]). You will need 400 ml of culture for 20 filters.
2. Collect bacteria by centrifugation and resuspend them in an equal volume of fresh LB + 10 m*M* $MgSO_4$. General methods for handling λ are found in this volume [13].
3. Label the filters and mark them asymmetrically, with a black ballpoint pen, on the side that will be in contact with the plaques.
4. Dip the filters in the bacterial cell suspension and allow them to air dry briefly.
5. Lay the filters on the surface of the plates containing plaques. Transfer the orientation marks to the agar plate. The techniques in step 4 of the alternate procedure can be employed.
6. Prepare additional filter copies, if desired. Be sure to transfer orientation marks from agar to filter. A light box is a useful aid here.
7. Lay copy filters, phage plaque side up, on fresh LB + Mg^{2+} plates and incubate, inverted, at 37° overnight.

During the overnight growth at 37° the plaques infect the growing *E. coli,* leading to a substantial amplification of phage DNA. After this amplification, it is usually not necessary to hybridize two sets of filters to avoid false positives.

8. Remove the filters from the plates, air dry for at least an hour and process filters as in step 16, omitting the 10% SDS treatment as described above. Store plates, inverted, sealed in Parafilm at 4°. The filters are ready for prehybridization (this volume [45]).

[45] Screening Colonies or Plaques with Radioactive Nucleic Acid Probes

By GEOFFREY M. WAHL and SHELBY L. BERGER

Colony or plaque hybridization is a technique for screening replicated material *in situ* on filters with labeled probes.[1-5] The probes most com-

[1] M. Grunstein and D. S. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **72,** 3961 (1975).
[2] M. Grunstein and J. Wallis, this series, Vol. 68, p. 379.
[3] W. D. Benton and R. W. Davis, *Science* **196,** 180 (1978).
[4] D. Hanahan and M. Meselson, *Gene* **10,** 63 (1980).
[5] D. Hanahan and M. Meselson, this series, Vol. 100, p. 333.

monly used are nucleic acids or antibodies. Here we will describe techniques for using nucleic acids to analyze libraries generated in either phages or plasmids. The use of antibodies for screening libraries can also be found in this volume [50, 51].

A library is a mixture of clones constructed by inserting either cDNA or fragments of genomic DNA into a suitable vector. The term *library* implies the existence of large numbers of different recombinants, only one or a few of which are of immediate interest to the investigator. The desired clone is located by performing the following steps: (1) transfected bacteria or phage are grown on master plates (or filters) and replica plated; (2) the original plates called *master plates* are preserved while the replicas, hereafter called *filters,* are processed; (3) phage are disrupted or bacteria are lysed *in situ* on filters; (4) DNA is bound to the filter while RNA is hydrolyzed; (5) the resulting partially denatured DNA is hybridized to sequences able to bind specifically to the desired insertions. (6) Because the configuration of DNA on the filter replicas matches the configuration of live bacteria or phage on the master plates, DNA on replicas which binds to the probe (so-called positive signals) can direct the investigator to the bacterial colony or phage plaque from which the DNA was derived; (7) the positive colony or plaque is then purified and grown in quantity for further analysis.

Chapters [44] and [18] describe steps 1–4 for plasmid or λ libraries and cosmid libraries, respectively. Here we will focus on steps 5–7.

Colony hybridization is a rapid but inexact procedure aimed at calling attention to clones worthy of serious consideration. Falsely positive clones are therefore not uncommon. To some extent these can be reduced by the following: (1) use both negative control filters and, if possible, positive control filters; (2) screen duplicate filters of each master plate; and (3) prepare probes carefully.

To satisfy the requirements of point 1, it is advisable to include clones containing the vector without an insert or containing an irrelevant insert. The latter is particularly important when fragments bearing homopolymer "tails," usually composed of dG on one strand and dC on the other, are screened; the GC-rich regions on either end of the insert can hybridize to GC-rich probes and cause spurious positive signals. Thus, the use of known negative recombinants acts as a means for detecting unwanted cross-hybridization of the probe to vector and host DNA (which are also present) and also serves to establish the intensity of a background signal, one that should be ignored. Since intensities are relative, a genuine positive signal is needed for comparison. If there are no known positive clones, one can always clone the probe itself and create a positive recombinant. Such engineered positive colonies or plaques are rarely perfect

The claims, as currently written, are drawn to library or libraries of polynucleotides comprising polynucleotides corresponding to the accessible regions of cellular chromatin obtained by the method of claim 3. Each of these claims is directed to a genus comprising any library of polynucleotides comprising polynucleotides corresponding to the accessible regions of cellular chromatin obtained by the method of claim 3. Note that absent an explicit definition in the specification of the term, a library is interpreted as "an unordered collection of clones (i.e., cloned DNA from a particular organism)" (see Biotech Life Science Dictionary, URL: http://biotech.icmb.utexas.edu/search/dict-search.phtml?title=library). Thus the number of clones of polynucleotides in each library may vary. Further, since the probes used in claim 3 may be a chemical, an enzyme or an antibody, each of which may react with, and thus mark, different polynucleotides, the claimed genus comprises different species of libraries comprising different polynucleotides.

A description of a genus may be achieved by means of a recitation of a representative number of species, falling within the scope of the genus, or by means of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus. Regents of the University of California v. Eli Lilly & Co., 119 F3d 1559, 1569, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). In the instant case, however, the specification does not describe the structure (i.e. the sequences of each clone of a library) of any species, nor does it describe any structural feature (i.e. the sequence of each clone in a library) common to the members of the genus. No common structural attributes identify the members of the genus. While the specification gives example of how to make a library (see pages 113-116), it does not describe the structure of the library or libraries made. The general knowledge and level of skill in

RELATED PROCEEDINGS APPENDIX

As noted above on page 2 of this Brief on Appeal and pursuant to 37 C.F.R. § 41.37(c)(i) and (c)(x), Appellant has filed a Notice of Appeal in USSN 10/083,682, which is a CIP of the instant application. Inasmuch as no briefs have yet been filed, nor decisions received in that appeal, no documents are submitted with this Appendix.